Determination and Thin Layer Chromatographic Confirmation of Identity of Aflatoxins B_1 and M_1 in Artificially Contaminated Beef Livers: Collaborative Study

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An international collaborative study involving 13 laboratories was conducted to test methods for the determination and thin layer chromatographic (TLC) confirmation of identity of aflatoxins B_1 and M_1 in beef liver. For the determination, each collaborator furnished fresh or frozen beef liver. Samples were artificially contaminated by adding solutions containing various concentrations of aflatoxins B_1 and M_1 (0.032-0.69 ng/g). Two TLC confirmation methods were tested with extracts obtained from the determination. Two measurement methods using 2-dimensional TLC were evaluated. In the first, sample extracts were compared directly with B_1 and M_1 standards on TLC plates; in the second, internal standards plus sample extracts were compared with B_1 and M_1 standards on the plates. Average within-laboratory coefficients of variation (CV) for the direct method were 26% for B_1 and 26% for M_1 compared with 24 and 26%, respectively, for the internal standard method. The average between-laboratory CV values were 39% for B_1 and 41% for M_1 by the direct method and 36% for B_1 and 39% for M_1 by the internal standard method. Recoveries ranged from 64 to 90% for B_1 and from 72 to 86% for M_1. These data indicate that the more convenient direct method was sufficient, and internal standards were unnecessary. An analysis of variance was calculated from combined sample data to determine components of variance. The within-laboratory CV values were 27.0 and 32.3% for B_1 and M_1, respectively, and the between-laboratory CV values were 47.1 and 53.2%, respectively. Both TLC confirmation methods gave satisfactory results and have been adopted official first action, along with the determination method.

Aflatoxin-contaminated agricultural commodities (corn, peanuts, and cottonseed) have occurred worldwide. Observation of aflatoxin M_1 in milk indicates that contaminated commodities have been fed to dairy animals and probably to other farm animals. Therefore, it is important to examine the edible tissues of farm animals and poultry with current sensitive methods to see if aflatoxin residues in meats are a potential problem. Analytical methods for determining aflatoxins in tissue have improved in sensitivity and accuracy since 1976. A methods evaluation (unpublished) by mycotoxin analysts in France, The Netherlands, and the United States resulted in the method of Stubblefield and Shotwell (1) being tested in an international AOAC/IUPAC collaborative study along with thin layer chromatographic (TLC) confirmation method **26.A15** (2) and the method of van Egmond and Stubblefield (3).

Obtaining samples for this study was a major problem not normally encountered in collaborative studies. Sufficient naturally contaminated beef liver was not available to provide collaborators with identical, duplicate samples. Also of major importance was the cost and difficulty of shipping frozen livers to collaborators throughout the world. In 1980, L. Stoloff (Mycotoxin General Referee, AOAC) and M. Jemmali (Mycotoxin Working Group Chairman, IUPAC) undertook a joint collaborative study (unpublished) to test the methods with freeze-dried, naturally contaminated liver powder. The results of this study did not give the precision required for recommendation to AOAC or IUPAC. Part of the problem was due to sample preparation, because changes in the liver caused by freeze-drying makes sufficient cleanup of extracts very difficult. Fluorescent impurities remain in extracts for TLC and obscure the aflatoxin zones (1). To circumvent this problem, standard solutions of aflatoxins B_1 and M_1 were

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The recommendation of the Associate Referee was approved by the General Referee and Committee C and was adopted by the Association. See *J. Assoc. Off. Anal. Chem.* 65, 374 (1982).

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provided to collaborators in the present study as samples, and collaborators provided fresh or frozen beef liver to be artificially contaminated. Restricting the study to fortified samples is not ideal, but it was a necessary compromise. We now report the study results.

Collaborative Study

Standard Aflatoxin B_1 and M_1 Solutions

Crystalline aflatoxins B_1 and M_1, with purity criteria previously reported (4, 5), were used to prepare stock solutions of 316.40 μg B_1/mL and 158.78 μg M_1/mL in acetonitrile. Concentrations were determined (in benzene–acetonitrile, 98 + 2 and 9 + 1, respectively) using extinction coefficients of 19 800 (B_1) and 18 815 (M_1) according to method **26.004–26.011** (2). Three standard solutions for TLC, containing 0.25 μg B_1, 0.25 μg M_1, and 0.25 μg B_1 + 0.25 μg M_1/mL in benzene–acetonitrile (9 + 1) were prepared and sealed in glass ampules.

Preparation of Solutions for Artificially Contaminating Beef Liver

Aliquots of B_1 (1.0 mL) and M_1 (1.0 mL) from stock solutions (see above) were added to 10 mL volumetric flasks to prepare 1:10 dilutions of stock B_1 (31.64 μg/mL) and stock M_1 (15.88 μg/mL) in acetone. Aliquots of the diluted stock solutions were added to 100 mL volumetric flasks to prepare the following sample solutions (acetone) for addition to uncontaminated and artificially contaminated samples: (a) 0.0 μg B_1 and 0.0 μg M_1/mL; (b) 3.2 ng B_1/mL; (c) 10.0 ng B_1 and 10.0 ng M_1/mL; (d) 30.0 ng B_1/mL; (e) 69.0 ng B_1 and 40.0 ng M_1/mL; (f) 40.0 ng B_1 and 20.0 ng M_1/mL; and (practice) 50.0 ng B_1 + 50.0 ng M_1/mL. Addition of 1.0 mL sample solution to 100 g blended liver gave aflatoxin concentrations of 0.00–0.69 ng/g. Sample solutions were dispensed in glass ampules, and ampules were sealed and randomly coded.

Methods

The method of Stubblefield and Shotwell (1) to determine B_1 and M_1 in animal tissue and the TFA–TLC confirmation method **26.A15** (overspot) (2) and van Egmond and Stubblefield method (3) (spray) were tested.

Description of Study

Sixteen collaborators each received the following items: 1 ampule each of the 3 standard solutions; 1 ampule of trifluoroacetic acid (TFA); 1 ampule of practice liver-contaminating solution; 12 ampules of liver-contaminating solutions (duplicates of spiking solutions numbered randomly); and a copy of the study instructions and report sheets. Each collaborator supplied fresh or frozen beef liver that was aflatoxin-free as determined by the quantitative method.

Aflatoxin concentrations were selected to test the methods at levels that might be encountered in edible meat tissues (0.03–0.7 ng/g). Collaborators were instructed to blend a sufficient quantity of uncontaminated beef liver until uniform, weigh 100 g into a 500 mL wide-mouth, glass-stopper Erlenmeyer flask, add 1 mL spiking solution, and mix thoroughly with a heavy glass rod in preparation for extraction. Collaborators were cautioned to prepare only the number of samples that they could analyze completely in 1 day. The practice spiking solution of stated concentration was included to familiarize analysts with the methods. Analysts were asked to use either or both TLC confirmatory methods and to return a photograph of each sample confirmatory plate with the report sheets. The collaborators were requested to quantitate each sample by the direct method (1) and by the internal standard (indirect) method. The latter consisted of overspotting the sample extract at the origin with 5 μL B_1 + M_1 standard solution and, after plate development and zone area measurement, deducting the internal standard. Both values were to be recorded on the report sheets. TLC plates with internal standard also served to locate B_1 and M_1 on the plates in relation to any interferences, and should be used for this purpose whether or not they are used for quantitation.

Aflatoxins B_1 and M_1 in Liver

Official First Action

26.C01 *Apparatus*

(a) *Wrist-action shaker.*—Burrell, or equiv.

(b) *Meat grinder.*—Waring blender, Model EP-1, and any manual food grinder.

(c) *Chromatographic columns.*—Glass column 30 × 1.0 (id) cm with porous polyethylene frit (35 μm) and Luer nylon stopcock (Bio-Rad Econo-columns No. 737-2260 and 732-9009, resp., or equiv. glass column).

(d) *Filter paper.*—32 cm, S & S No. 588, or equiv. rapid flow, high wet-strength paper; and 24 cm, S & S No. 560, or Whatman 2V or equiv., medium flow paper.

(e) *Thin layer plates.*—10 × 10 cm com. prepoured, 0.25 mm thickness, glass plates (hand-cut

from 20 × 20 cm) (E. Merck silica gel 60, No. 5763 or Macherey, Nagel Sil G-25 HR), or prep. in laboratory as follows: 10 × 10 or 20 × 20 cm plates coated with 0.25–0.5 mm (wet thickness) layer of Macherey-Nagel GHR silica gel for TLC (Macherey, Nagel & Co., D-5160 Duren, GFR; distributed by Brinkmann Instruments, Inc.) dried 1 h at 105° or Adsorbosil-1 silica gel for TLC (Applied Science Laboratories, Inc.), or equiv.

26.C02 *Reagents*

(a) *Solvents.*—Reagent grade, distd in glass. Glacial HOAc, acetone, CH_3CN, benzene, $CHCl_3$ (0.75% EtOH), CH_2Cl_2, ether (≤0.1% EtOH, peroxide-free), hexane (68–69°), isopropanol, and toluene.

(b) *Citric acid soln.*—20%. Dissolve 200 g citric acid monohydrate in 1 L H_2O.

(c) *Silica gel for column chromatography.*—E. Merck silica gel 60 (No. 7734), 0.063–0.200 mm (70–230 mesh), or equiv. Stir 1 h in MeOH, filter, and treat similarly with $CHCl_3$. Activate by drying 1 hr at 105°. Add H_2O, 1 mL/100 g, seal, shake until thoroly mixed, and store ≥15 h in air-tight container.

(d) *Sodium sulfate.*—Anhyd., granular.

(e) *Diatomaceous earth.*—Hyflo Super-Gel.

(f) *Aflatoxin reference stds.*—Prep. as in **26.004–26.011** to contain 0.25 μg aflatoxin B_1 and M_1/mL in benzene–CH_3CN (9 + 1) for either visual or densitometric analysis. If aflatoxins G_1, B_2, and/or G_2 are needed, prep. G_1 at 0.25 μg/mL and B_2 and G_2 at 0.05 μg/mL. Store stds in 1 dram vials fitted with Teflon-lined screw caps at 0°F when not in use.

26.C03 *Extraction*

Blend or grind meat tissue until homogeneous. Weigh 100 g mixt. into 500 mL wide-mouth, g-s erlenmeyer (or equiv.). Add 10 mL citric acid soln and mix thoroly with 30 cm × 1 cm glass stirring rod. After 5 min, stir again, and mix with 20 g diat. earth. Add 200 mL CH_2Cl_2 and stir to remove excess solids from rod. Shake flask vigorously on wrist-action shaker (setting 5 on a Burrell) for 30 min. Filter mixt. thru fast flow paper into 300 mL erlenmeyer contg 10 g Na_2SO_4. Close filter top and compress entire filter against funnel to obtain max. filtrate vol. Gently swirl flask intermittently ca 2 min and refilter contents thru medium flow paper into 250 mL graduate and record vol. (cover funnel with watch glass to prevent evapn of solv). Evap. filtrate in 500 mL r-b flask, under vac., to near dryness and save for column chromatgy.

26.C04 *Column Chromatography*

Fill column half full with CH_2Cl_2 and add 2.0 g silica gel. Add 3–4 mL CH_2Cl_2 and slurry silica with stainless steel rod (ca 0.32 cm diam.). Drain CH_2Cl_2 to settle silica and rinse silica off column sides with CH_2Cl_2. Add 2 g Na_2SO_4 to supernate solv. above silica gel to cap column and drain excess CH_2Cl_2 to ca 1 cm above column packing.

Redissolve concd filtrate in ca 25 mL CH_2Cl_2, add to column, rinse r-b flask and column with addnl CH_2Cl_2, and drain entire soln thru column by gravity. If flow rate slows, stir Na_2SO_4 gently. When filtrate reaches Na_2SO_4, rinse column sides with CH_2Cl_2 and drain similarly. Wash column with 25 mL toluene–HOAc (9 + 1), 25 mL hexane, and 25 mL hexane–ether–CH_3CN (6 + 3 + 1) and discard washes. Elute aflatoxins with 40 mL CH_2Cl_2–acetone (4 + 1) and evap. eluate to near dryness in vac. or on steam bath. Quant. transfer ext with $CHCl_3$ or CH_2Cl_2 rinses to 1-dram vial with Teflon-lined screw cap. Evap. to dryness under N on heat source, but avoid overheating of dry ext. Save for TLC.

26.C05 *Visual and Densitometric Analysis*

Add 100 μL benzene–CH_3CN (9 + 1) to sample residue in vial from **26.C04,** cap vial, and mix vigorously ca 1 min, preferably on vortex mixer. After TLC analysis, reserve remaining ext in freezer for confirmation of identity.

See Figure **26:01** for spotting and scoring patterns of 2-dimensional TLC plates, except dimensions for 20 × 20 cm plate, direction 1, bottom to top are as follows: 2, 11, 1, 1, 1, 4 cm and dimensions for direction 2, left to right are 2, 12, 6 cm; similarly for 10 × 10 cm plate: direction 1: 1.5, 4.5, 1, 1, 1, 1 cm, and direction 2: 1.5, 6.5, 2 cm. Spot 20 μL aliquot of sample ext on sample spot and either 1.5, 0.5, 1.0, and 1.5 ng of ref. std (visual) or 2.5, 1.25, 1.25, and 2.5 ng of ref. (densitometric). Develop plate in ether–MeOH–H_2O (95 + 4 + 1) in first direction (see also **26.013**). When solv. reaches score line, remove plate, air-dry, heat in forced air oven at 50° (ca 2 min), cool plate, and redevelop in second direction in $CHCl_3$–acetone–isopropanol (87 + 10 + 3) to score line. Quantitate visually or densitometrically as in **26.074** and calc. concn of B_1 or M_1 as:

$$\mu g/kg = (S \times Y \times V)/(X \times W)$$

where S = μL aflatoxin ref. std equal to unknown; Y = concn of ref. std, μg/mL; V = μL of final diln of sample ext; X = μL sample ext spot-

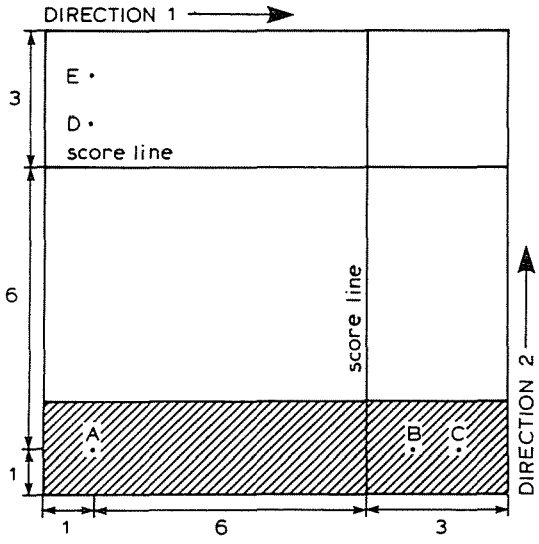

Figure 26:C1. Schematic representation of thin layer chromatogram for confirmation of identity of aflatoxins B_1 and M_1. A = spotting place for sample extract; B and D = spotting place for M_1 standard; C and E = spotting place for B_1 standard.

ted giving fluorescent intensity equal to *S* (ref. std); *W* = (100 g or mL × filtrate vol.)/200.

Confirmation of Aflatoxins B_1 and M_1 in Liver

Official First Action

26.C06 ***Reagents***

(a) *Solvents.*—$CHCl_3$ (≤0.75% EtOH), acetone, isopropanol, and hexane.

(b) *TFA-hexane spray.*—(1 + 4). Mix 1 vol. of trifluoroacetic acid (TFA, ≥95% pure) with 4 vols of hexane. Prep. fresh daily.

(c) *Aflatoxin std solns.*—Prep. sep. std solns of aflatoxins B_1 and M_1 (0.25 μg/mL each) in CH_3CN–benzene (1 + 9) or $CHCl_3$.

(d) *TLC plates.*—0.25 mm thick layer of Macherey-Nagel Sil-G-25HR silica gel (Macherey, Nagel & Co., D-5160, Duren, GFR, distributed by Brinkmann Instruments, Inc.) or Merck Kieselgel 60 on 10 × 10 cm plates, self-cut from 20 × 20 cm plates.

26.C07 ***Apparatus***

(a) *UV illumination cabinet.*—365 nm.

(b) *Disposable capillary pipets.*—10 and 20 μL, or microsyringes.

(c) *Spray unit for thin layer chromatography.*—Low vol. capacity (5–20 mL).

(d) *Air dryer.*—Unit capable of providing stream of warm air (40–50°) to evap. solv. from TLC plates.

26.C08 ***Thin Layer Chromatography***

Score 2 straight lines on 10 × 10 cm TLC plate at right angles (3 cm in from each edge) (see Figure **26:C1**) to limit migration of developing solv. fronts. Spot following solns on plate, using capillary pipets or microsyringes:

(a) Vol. of sample ext equal to vol. used for quantitation on point A (normally ca 20 μL).

(b) Vol. of std soln contg ca 2.5 ng M_1 on points B and D.

(c) Vol. of std soln contg ca 2.5 ng B_1 on points C and E.

Develop plate in first direction with isopropanol–acetone–$CHCl_3$ ((3 + 10 + 87) for Macherey-Nagel TLC plates or (8 + 10 + 82) for Merck TLC plates) (see Figure **26:C1**), until solv. front reaches solv. limit line. Dry plate after development 5–10 min with stream of warm air to evap. solv. completely (check odor). Spray TFA–hexane soln from a distance of 5–10 cm along band (ca 2 cm wide), covering points A, B, and C (indicated by hatched area in Figure **26:C1**) until plate is thoroly sprayed (ca 2 mL spraying reagent). After hexane has evapd, cover TLC plate with warm, clean, glass plate (75°) and immediately heat 6–8 min in 75° oven with TLC plate on oven floor. Cool 1 min on cold surface, evap. excess TFA with stream of air, and develop in second direction with isopropanol–acetone–

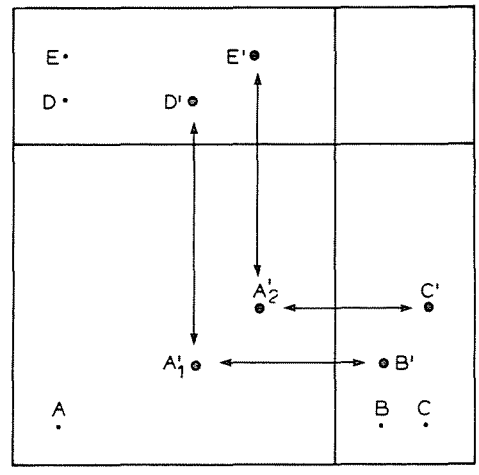

Figure 26:C2. Schematic representation of thin layer chromatogram after confirmation chromatography. A'_1 = M_1 derivative from sample; A'_2 = B_1 derivative from sample; B′ = M_1 derivative from standard; C′ = B_1 derivative from standard; D′ = M_1 from standard; E′ = B_1 from standard.

$CHCl_3$ ((6 + 10 + 84) for Macherey-Nagel TLC plates or (12 + 10 + 78) for Merck TLC plates).

Examine plate under longwave UV light (365 nm) and check for following fluorescent zones (see Figure **26:C2**):

(a) Appearance of blue fluorescent spots D′ and E′ of std aflatoxins M_1 and B_1, resp., originating from D and E (migration in direction 1).

(b) Appearance of blue fluorescent spots B′ and C′ of TFA derivatives of aflatoxins M_1 and B_1, resp., originating from std solns spotted at B and C (migration in direction 2).

(c) Appearance of blue fluorescent spots A_1' and/or A_2' originating from ext spotted at A, with R_f values matching those of spots B′ and C′, resp.

Identity of aflatoxin B_1 in ext is confirmed when R_f values of B_1 derivative from Sample (A_2') and B_1 std (C′) match. Similarly, identity of aflatoxin M_1 in ext is confirmed when R_f values of M_1 derivative from Sample (A_1') and M_1 std (B′) match.

Results

Individual values were omitted from calculations according to Dixon's test for outliers at the 0.05 level (6). For statistical calculations, the second value submitted was substituted for the outlier to maintain balance in the analysis of variance. Similar treatment was given to spilled Sample 5 for Collaborator 14. Values for Collaborators 2 and 16 were not included in the calculations because the composite data for each exceeded the lower limit of Youden's ranking test (7). Collaborators 7 and 13 were borderline in the same test, but both are included in the calculations. Collaborators 8 and 10 did not submit results by both the indirect and direct measurement procedures, so their data were not used in analysis of variance calculations.

The results reported for aflatoxins B_1 and M_1 in artificially contaminated beef liver are given in Table 1. The statistical summary for these results is presented in Table 2. Means for both B_1 and M_1 obtained by the internal standard method were lower than those from the direct method. However, there is no significant statistical difference between them. Although a TLC plate with an internal standard should always be prepared to establish the chromatographic separation of the aflatoxins from interferences, quantitation of this plate does not appear to improve accuracy and precision of the total measurement. Recoveries for aflatoxin B_1 by the direct method were 64–90%. The statistical data (% recovery) for Sample 4 strongly suggest that an error occurred in sample solution preparation by the Associate Referee. Based on the other recoveries, this sample probably contained 0.22–0.23 ng B_1/g instead of the intended 0.30 ng/g. Recoveries for aflatoxin M_1 by the direct method ranged from 72 to 86%. The low value was found at the 0.10 ng/g level, the lowest level tested. Coefficients of variation (CV) for B_1 and M_1 were 31–54% by the direct method. These CV values are comparable to those calculated in other aflatoxin collaborative studies. Of the 352 determinations (including Collaborators 8 and 10), there were 7 false negatives for B_1 and 2 false negatives for M_1, all at the lowest levels tested (0.03 ng B_1/g and 0.10 ng M_1/g) (Table 1); most of these were from plates for which subtraction of the internal standard resulted in a small negative value. If only the results obtained by direct measurement are used, only 2 false negatives (B_1, Sample 2, Collaborator 10) were reported.

The results for uncontaminated beef liver are given in Table 3. There were 6 aflatoxin B_1 false positives for 42 total observations, of which only 2 were by direct determination. The identity of 1 of these was not confirmed. Of the 130 M_1 observations, there were 16 false positives. Only 4 (0.1 ng/g) were reported by the direct method; however, the identities of all 4 were confirmed. Most of the fluorescent contaminants present in the liver extracts occur in the M_1 area of the TLC plate. Either those collaborators with false positives accidentally contaminated their samples or, if not, they should experiment with solvent systems to better resolve M_1 from contaminants. Also, livers used for spiking could have been contaminated if not checked beforehand. Obvious large differences in TLC interferences were observed based on the photographs. This was expected because of the different animal breeds, ages, gender, and feed rations involved for the beef livers. Although these factors prevented identical samples from being tested, they did provide for realistic samples as would be encountered in practice, except that all were artificially contaminated.

The precision estimates calculated to compare measurement methods for B_1 and M_1 on an individual sample basis are given in Table 4. The within-laboratory coefficient of variation (CV_o) is the repeatability, and the between-laboratory coefficient of variation (CV_x) is the reproducibility. Averages for B_1 were 26% (CV_o) and 39% (CV_x) by the direct method, with very little difference (24 and 36%, respectively) by the internal standard method. The averages for aflatoxin M_1

Table 1. Collaborative study results (ng/g × 10^2) for determination of aflatoxins B_1 and M_1 in artificially contaminated beef liver [a]

		Sample 2		Sample 3				Sample 4		Sample 5				Sample 6			
Coll.	Meth [b]	B_1		B_1		M_1		B_1		B_1		M_1		B_1		M_1	
1	I	1.7	1.8	9.6	5.9	3.4	2.9	20.8	28.7	65.3	70.6	28.0	20.0	33.1	37.9	9.5	9.5
	D	2.2	2.4	8.7	5.9	3.4	2.9	25.0	32.8	70.0	70.6	28.0	25.2	35.5	37.9	11.8	11.8
2 [c]	D	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(10.0)	(29.0)	(24.0)	(22.0)	(17.0)	(0)	(11.0)	(0)	(7.0)
3	I	2.0	2.0	7.0	8.0	3.0	7.0	24.0	23.0	60.0	89.0	28.0	41.0	28.0	46.0	14.0	19.0
	D	3.0	2.0	10.0	8.0	6.0	10.0	23.0	21.0	61.0	91.0	29.0	45.0	31.0	44.0	14.0	20.0
4	I	3.0	2.0	8.0	7.0	5.0	7.0	17.0	22.0	51.0	83.0	25.0	17.0	49.0	48.0	10.0	19.0
	D	3.0	2.0	8.0	9.0	5.0	5.0	17.0	22.0	59.0	100.0	25.0	25.0	49.0	32.0	10.0	19.0
5	I	1.4	0.4	5.0	5.6	5.9	4.9	29.0	19.0	75.0	57.0	40.0	25.0	42.0	35.0	16.0	15.0
	D	1.7	1.6	6.3	5.2	7.3	7.8	29.0	19.0	83.0	61.0	41.0	29.0	40.0	41.0	17.0	17.0
6	I	2.5	4.0	8.0	10.0	7.0	8.0	25.0	24.0	81.0	102.0	51.0	50.0	40.0	48.0	21.0	27.0
	D	2.5	4.0	8.0	10.0	7.0	8.0	25.0	24.0	81.0	102.0	51.0	50.0	40.0	48.0	21.0	27.0
7	I	3.7	2.0	6.0	3.6	2.5	6.5	11.0	10.1	46.5	31.2	28.5	21.6	30.4	20.2	6.5	12.2
	D	4.4	5.6	0	3.4	1.8	4.0	12.0	13.7	44.5	25.8	26.2	20.3	30.1	21.4	7.7	9.3
8 [d]	I	0	(3.0) [e]	(3.0) [e]	(3.0) [e]	0	0	20.0	3.3	30.0	30.0	7.0	5.0	8.0	7.0	8.0	3.0
10 [d]	D	0	0	5.0	5.0	15.0	13.0	9.0	13.0	20.0	60.0	34.0	70.0	9.0	9.0	21.0	21.0
11	I	5.0	8.0	10.0	13.0	4.0	13.0	23.0	20.0	89.0	89.0	53.0	50.0	44.0	33.0	20.0	26.0
	D	4.0	4.0	9.0	10.0	6.0	10.0	26.0	26.0	77.0	82.0	35.0	48.0	48.0	33.0	22.0	15.0
13	I	0	0	6.0	3.0	2.0	1.0	8.0	7.0	26.0	54.0	20.0	22.0	31.0	23.0	7.0	8.0
	D	1.0	(3.0) [e]	7.0	8.0	4.0	6.0	9.0	7.0	32.0	56.0	25.0	26.0	32.0	27.0	12.0	11.0
14	I	1.2	3.3	5.0	5.4	5.5	10.5	12.5	12.5	42.0	(27.3) [f]	23.1	(17.3) [f]	18.1	16.0	16.1	14.7
	D	2.2	4.9	8.6	4.2	8.5	12.4	13.8	17.1	54.6	(21.8) [f]	34.3	(14.1) [f]	28.9	19.7	14.2	14.0
15	I	(11.0) [g]	(3.0) [e]	11.0	8.0	17.0	9.0	10.0	26.0	20.0	33.0	17.0	30.0	38.0	31.0	19.0	22.0
	D	(11.0) [g]	(3.0) [e]	12.0	7.0	(19.0) [g]	9.0	11.0	25.0	29.0	39.0	22.0	31.0	31.0	26.0	19.0	22.0
16 [c]	D	(0)	(0)	(0)	(0)	(3.0) [e]	(10.0)	(3.0) [e]	(3.0) [e]	(3.0) [e]	(10.0)	(35.0)	(15.0)	(0)	(0)	(10.0)	(0)

[a] As determined by the method of Stubblefield and Shotwell (1).
[b] Method of measurement: (D) = sample zones directly compared with standards; (I) = internal standard spotted with sample extract and compared with standards.
[c] Values omitted from calculations after applying Youden's ranking test (7).
[d] Collaborator submitted either direct or indirect data only. Values not included in analysis of variance.
[e] Collaborator reported trace. Trace was taken as 0.03 ng/g for statistical purposes.
[f] Sample was spilled. Values not used in calculations.
[g] Values omitted from calculations as outliers by Dixon's test (6).